

Figure 1. Overlap-extension-PCR fragment
with purD deletion

Overlap-extension-PCR fragment
with recA deletion

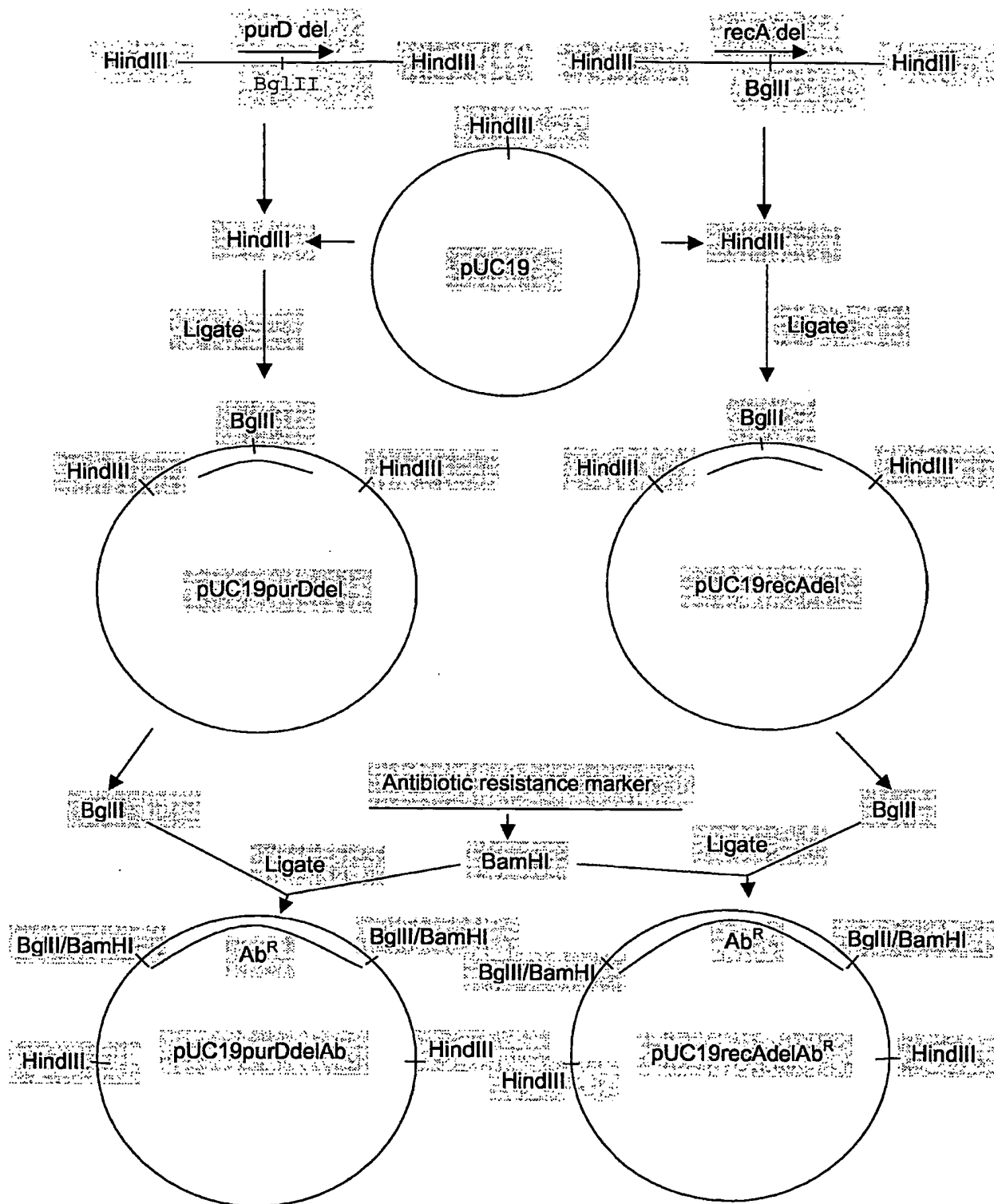


Figure 2A.

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1  GTTCGACCAA ACGGCTTGTT GTGCGGTGAA ACATAGCACT CCTTGTGGCG TGGCTTTAGA TGATGATATT TTGCAAGCGT
   >>.....F5.....>>          CTTAAGCTTGGAG>>.....F13.....>>
                                   -----
                                   HindIII

81  ACCAAAAAGC ACACGACTGC GACCCGATTT CGATTTTGGG TGGCATTGTA ACTTTTAATA AAAAAGTAAC AAAAGCAGTG
161 GCAGAAAAAT GTAACGAGAT TTTCCTTGAA ATCGTTGCTG CACCGAGCTT TGAGCCAGAG GCTTTGGAAG TTTTGTCTAA
241 AAAGAAAAAT TTGCGCGTGA TTGAAGTTAA AAATCCATTA AGCGATAAAA TGCAACTCGT GCAAGTAGAT GCGGAGTTGC
321 TCGTGCAAGA AATCGACAAA TCGTTTAGCA ATGATTTTAA AGTAGTAACC GAAAAACAAC CTACCGAAAA GCAACTTTCT
401 GATTTTGAAT TTGCCATGAA AGTAGTGAAA CATGTAAAGA GCAATGCCAT CGTGGTTGCC ACAAACGGAC AAGCTCTAGG
481 CGTGGGCACA GCGGAGACTA ATCGTATTTG GGCAGCACAG CAGGCGATTC AGCGTGCAAA GGAAAAACA CAAGAAAAATC
561 TAGTTTGGC TTCCGATGCC TTTTCCCAT TCAGAGATGT GGTAGATTAT GCAGCACAAG AAGGCATTAC AGCCTTGATT
641 CACCCAGGAG GAAGCATGCG CGACCAAGAG AGCATAGACG CGGCTAATGA ACACGGAATC CCGATGATCA TCAGCGGTAT
721 GAGACATTTC TTACATTAAT TCAAAAAATC TAAACAATAA TTATCAATAA TTCTAAAACA CAATAAGTAT GAATGCAAAAT
                                   >>...purD...>

801 GATTACAAAA AAATACTCAT CGTAGGAAAC GCGCAAGAG AACACGCCAT CGGGTGGAAA ATTAACCAAG ACCACCCCTC
   >.....purD.....>

881 TTGCGAGCTT TTCTTGGCG CAGGAAACGC TGGAAACGAA CAAATTGGAA AAAACATCGT AGCTGAATCT AATTATGGCT
   >.....purD.....>
                                   <<.....OE-R.....<<AGATCTGGCGCTACGCTAGAAG
                                   -----
                                   BglII

961 TAATGCTTTT TGCTCAACAA AATGATATAG ACTTAACGAT TGTAGGTCCA GAAGCAGAAT TGGTAGAAGG TATTGTAGAC
   >.....purD.....>

1041 TTGTTTGAAT CCAATCAATT AAGAATTTT GGTCCAGATA AGCGTGCGGC TAAATTGGAA GGCAGCAAGG CTTTGGCCAA
   >.....purD.....>

1121 AGATTTTATG GAGAAATACG GCGTGCGCAC GGCTTTTGCC AAAAGTTTCA ACAATTTTGT AGACGCTAGA GATTATGTAA
   >.....purD.....>

1201 AAGAGCTCAC GCAATTCCTT ATCGTGATCA AAGCCAGTGG CTGGCAGCA GGAAAAGGTG TGATCATCGT GCANTACAA
   >.....purD.....>

1281 CTTGAAGCCG AAATACTTT GCGCAAAATC ATGGAAGACA AAACCTTTGG CGAAGCAGGC AACGAGGTG TAATCGAGGA
   >.....purD.....>

1361 ATACTTAAAA GGTGTGGAAG TTTCTGTGCT TTCTATCTTT AACCATAAAG AAATTAAAC TTTCTTGCTT GTAAAAGACC
   >.....purD.....>

1441 ACAAGAAAAAT CGGAAAAGGC GAAACAGGAC TCAACACGGG CGGAATGGGC GTAGTGGCTC CTAACCCGCA TTTTACCGAT
   >.....purD.....>

1521 GAGCACATGA AGGAGTTTGA GAAAAACATT TTGCTCCCAA CAAAAAAGG GCTCTTGGCA GAAAAATGC ATTTGTCAGG
   >.....purD.....>

1601 CATTATTTTC TTTGGGCTTA TGATTACCGA GCATGGTATT TATCTATTGG AATACAACAT GCGATTTGGC GACCCAGAAA
   >.....purD.....>

1681 CCGAAGCACT TTTGCCTTGA ATGGAGAATG ATTTAGTAGC CCTCATCGAT TCCGCAATAC ACCAGCAAGA CATTTGAATT
   >.....purD.....>

1761 AAATGAAAAA ACGAACATGC TTGCTGTGTA GTAATGGCGA GCGGTGGCTA CCCAGGCACT TACGAAACTG GTTTTGAAT
   >.....purD.....>
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1841 CCGAGGATTG AACAAAGTTG ATGTTCCCGT ATTTATTGCA GGAGCCAGAG AAGAAAGTGG AAAAATCTAC ACCACAGGCG
>.....*purD*.....>

1921 GGCGCGTGCT CAATGTGGTG GGAAGTGGCG CTACGCTAGA AGAAGCCAGA AAAGTGGCTT ACGAAAATAT CCATAAAATC
>.....*purD*.....>
GAGATCTGG>>.....OE-F.....>>

BglII

2001 AATTTTGATT ATGAATATTA TCGCGAAGAC ATCGGGAAGA TATAATCTCG CTGATTTTTA ACCAAAACAT ATTTAAAAAC
>.....*purD*.....>>

2081 GCTTTTGTTA CTTTATATAA CAAAGCGGTT TTTCTATTTT TGTGCCACTA TAACATGATT TAACCCATGA AAAAAATACT

2161 AAAAAATACT ATTTTCTAC TGCTCATTCC TTGGGTTTAT GCCTGATTT TAATCTTTAT AAATCCACCT ATCACCATTA

2241 CACAGCTGAG CAATTTATCT TATGGTTTCT CCAGAACACA GCTCGCTTAT GATGAAATC CGGCTAGTGC TAAATGGGCT

2321 GTAATTGCAG CAGAAGACCA GAATTTTGCC ATTCATAATG GCTTTGATTT TAAAGAAATT AAAACCGCCT ACGAGAAAAA

2401 CAAAGCGGGC AAGAAATGTC GTGGCGGGAG CACCCTTTCG CAACAACTG CCAAAAATGT ATTTTGTGG CAAGGGCGCA

2481 CTTGGATTAG AAAAGGATTG GAAACCTACT GCACCTTTAT CATCGAACG CTGTGGAGCA AGGAGCGTAT TTTGCAAGTT

2561 TACCTCAACA ATGCCGAAT GGGCAAAGGC GTTTATGGCA TAGAGGCAGC GGCGCAATAT TATTTAAGA AAAACGCCTC

2641 ACAGCTCAGC CCTACCGAGA CGGCACGCAT CATTCCTGCG CTGCCCAATC CCAAAAAATA CAATNTAAAC CCGCCAAGTG

2721 CCTACATCTC AAAACGCGGA CAATGGATTC TGCGCCAGT GCGAACTTG AAAGGCGATA GGGCTCTGAG CGAGATTGTG

2801 AACACGCCCT AACGCCTGCC TCAACTCTTT GCACACAGTT TACCAACTCT CTGCGAAGAG TTCACAACT CTTCGCACAC

2881 ACTTCCCAA GTCTTTGCAA AGAGTTGGGA GATACTTAGG CACAAAAAA AGGAACCTCA TGAATAGAGG TTCCCTCTTC

2961 CTTAAAAGGA ATAAATAATA ATGTTTTTTA AGCTTTAGGC TTGGCTACTT TTTCAAAGCC TGCTGCCTTC ATGCTATCTA

HindIII

3041 GGATACGCTT GCCTGGGCGG TAGTTTACGC CTACCTTTT GATTAGCCG GAATGAAAAT CTTTCTCTGT ATCTGCCGCT
<<.....R8.....<

3121 CCACTGCTTA AAGTGGCATA GAGCGAGCCA AGCTTATCTA AACGAACGAT TTTGCCGCT GCCAAGGCGT CTGAATTAC
<R8.<<AAGCTTAAG
----- HindIII -----
HindIII

3201 ATTCTCTAGC GCAATGATAA CGCCACGAAT ATCTGCCCTG CTGAGTGCG AAAACTTCTC GATTGCTTA ACGAGCTGGT

3281 CTATATCCAT TTCTCCATCG CTTGCCACCA CGGCATAGTA TTTTGTGGC TCCCCTGGCT TGCTTGGGTT TCTACGCTGA

3361 ATTACATTGT ATTTATGCT CATAATTACT CTATTTTAA TAGCCTCCCG ATGGATATAA AGTTACGCTA CAATTAGGGT

3441 CTCCATAAGC AAATCTATAC CCCTCTCTTT CATATTCCCT TCTCATTCTT CTGCTCCAT CTCTCAAGGC ATCCGCTCTA

3521 TTAGTGCTAT ACCCCTCCTG AAGAAATGTG TCTGCACTTG AAGAAGAATA TGAAGAGCTA TGAGAATCGT GCAACATAGT

3601 CCAAGCTCCA TCTTGAGCTA TAACATTTGC ATGACATGTA ACACCTATAG TATAATAAAA TCTCCTAGGA GGTGTGTGTT

3681 CACCACCACC TCCAGAGCTA CTACTTTTTT TACATTGTCC ATTTTGGTTA GCATGATTTT GTCCGCCATC ACTTACTAAC

3761 TTCTTAGCTT CTGCTAAGGC TTTTCTCTT GCTTCTTTT CAGCATCTGC TTGGCTAATT CCACTCACTG CTGTAGCTGT

3841 CGCTTCTTTT TTATAGTTTA CCGAGGTTCC ATAATAGCCA CTACTACAAT TGTTCCTTGT AAAGTTTTTA TAAAAGATT

3921 GAGTTTGTGT TGAGGTGTAC CCTCCGAAAC CTTTACTTC TACAGTAAAG GTAGAACTCC CCATGCTTAC GGGGAAGGTG

4001 GCGATAGTAT ACGATTGCCC TGCCGGCATT TGTTTTACTT GATACACTCC ATCTCCTCCC ACTTCTATGC TTGCCGTAA

4081 ATTACCACTA CCGCTAAAAG AGCCTTCTGC TATTTTITAGT GTTAAATCAT TTATATCCCC TCCTTGTCCT TTGCAGAAG

4161 CTTTGTAC ACTTACAGCA TCATAAGCTC CTTTCCATT GGTATAAGGT ATTTATATGG CCAAAC

Figure 2B.

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1  TAAAGCTGTA AWTGCTATA AACGCCCTTT AGGATAAAAT CTGCCATTTT TTGCAGTATT TTWATAGCTA AAATTTAGAA
   >>.....FrecAOR1.....>>

81  AACACCATCT CGAGTAAAGG AGCGTGTAGT GCTCGCCATC GTTGAGCGAT TGCCACACCT CAATTGATTT GGGCGAATAC
   CTTAAGCTT>>.....F6.....>>
      -----
      HindIII

161  TTGAAATAAA TGGCATCTTC TAGCGACACA TTTTGCAGCAG AAATCATGCA AAAAGCCCCG CATAAAAAGC TGAATAAAAA
241  WGCTAWTYTT CTGTGTTAAA AAAACTCATA AATTCCCCCA AATATAGAAA TATTCTGTGA AAAGTTGCAA TTTATTAACA
   <<....<

321  CTATGTGCTT GCTTTTAATG AAAAAAGTAG ATTATTTTTC CGAATCCGAA AGTTTATTTA CGCCCCATCC GATGCCTAGT
   <..FrecA-4...<<

401  CCCMSCGATA GCCATGATTA ATACAAATAC AATTAAATCA WATTTTTCMC MTWWACCATA GCACAACACT TGCTAGCTCA
481  ACGAGTACTA GAGTGGTAAA AAGGATTTTT TGACGATTAT TCATGATTTT ATTTTCTCA AAGGTAAATA TTTTAAACCA
561  TAATTTTACA AATCTTAAAA TCTATTTAAA TAATAGAGAA ACCAGAAAAA AATCGTATTT TTACGGAATG AATAAATGT
641  TACAAGTAGG CGATAAAATG CCCGATTTCA AAGGTGTAGA CCAATTGGG AAGGAGCATT CATCTGCCGA TTTCAAAAAT
721  CAGAAATTAG TCGTTTTTTT CTACCCAAA GCCAGTACGC CAGGTTGCAC GGCAGAGGCT TGCAACATCA ACGATAATCT
801  TGATGCGCTA AAAGCACAAG GCTACCAAGT GATAGGCGTG AGTGCAGATT CGGTAGAAAA ACAACGAAAA TTCAGTGATA
881  AATACGATTT TAAATCCCT GTGATTGCCG ATGTGGATAA GAAAATTATT GAAGCATTG GCGTGTGGG CGAAAAGAAA
961  TTCATGGGTA AAACCTATGA CGGAATTCAT CGTACGACAT TCATTATTGA TGAAAACGGA GTGGTGGAGC GCGTGATAGA
   >>.....F7.....>>
      -----
      EcoRI

1041  AAAAGTGAAA ACAAAGATC ATACCAATCA AATTTTAAAT TCAGAAAAAT AAAAATATGA GCGAAATAGA CGAAGCGAAA
   >>.....recA.....>>

1121  AGGAAAGCAC TCCAGTAGT GCTTGATAAA ATGGACAAAA GCTATGGTAA AGGTGCCGTG ATGATGATGG GCGACAAAGC
   >.....recA.....>
   <<.....OER1.....<<

1201  CATAGACGAA AATATCCAG TAATCCCTAC GGGGTCTCTA GGTTTAGATT TAGCCTTGGG CGTGGGAGGG TATCCGCGCG
   >.....recA.....>
   <CGAGATCTCGTGGTGGGT
      -----
      BglIII

1281  GTAGAATCGT GGAGATTAC GGTCCAGAAT CTTCTGGTAA AACCATTG GCAATTCATG CCATTGCCGA AGCTCAAAAG
   >.....recA.....>

1361  TCTGGCGGAA TTGCAGCTTT CATCGATGCA GAGCAGCAT TTGATAGATA TTACGCAGAA AAATTAGGCG TAGATGTTGA
   >.....recA.....>

1441  GCATTTAATT ATCTCTCAGC CAGATAATGG GGAGCAAGCT TTAGAAATTG CCGATAACTT AATCCGTCA GGTGCAATTG
   >.....recA.....>
      -----
      HindIII

1521  ATATTATTGT AATCGATTG GTAGCGGCTT TAACGCCAAA GTCGGAATC GACGGAGATA TGGGCGATT CAAAATGGGA
   >.....recA.....>

1601  TTGCAAGCGC GTTTGATGC TCAAGCCTG AGAAAGCTCA CGGGAACAT CAATAAAACC AAATGTACTG CTATTTTCAT
   >.....recA.....>

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1681 CAACCAATTG AGAGAGAAAA TCGGTGTGAT GTTCGGTAGT CCAGAAACCA CAACGGGTGG TAATGCACTT AAATCTATG
>.....*recA*.....>

1761 CATCGGTGCG TCTAGACATT CGTCGTCTA CTCAGATTAA AGATGGGAAC GATGTCATCG GAACTTGAC TCGCGTAAAA
>.....*recA*.....>

1841 GTAGTGA AAA ACAAAGTAGC TCCGCCATTC CGTAGTCAG AATTCGACAT TATGTATGGC GAAGGAATCT CTAAAGCAGG
>.....*recA*.....>

BcoRI

1921 CGAGATTTTA GACATTGCTA CCGATTAGA AATCGTGAAA AAAAGTGGCT CTTGGTATTC TTATGCAGAT ACTAACTAG
>.....*recA*.....>

2001 GACAAGGGCG AGATGCCGTG CGTGC GTAT TGAAGATAA TCCAGAATTA GCCGAAGAAT TAGAAGAGAA AATTAAGAA
>.....*recA*.....>
CGAGATCT>>.....OEF1.....>>

BglII

2081 GAATTAGAGA AAAATAGAT TTTTAGTTT TTTAATTAA ACGAAAAATC CGTTCACTTT GTTGAACGGA TTTTTTTATG
>.....*recA*.....>>

2161 CTTGAATGAA TTTATTCCA ATGGATTGAA TAGCCATGCA CTTTAAATC TTCGCTATCA TAAGTGATTT CTTTGTGCGT

2241 GTTGGGATAG CAAACTTTAA GTCCTGCGTA TTTGGCAATG GCATGTCTG CGGCAATGTC CAAAAGTTT ACAGGTCTAA

2321 AGCGGGTGTA CTCCTAGACC CACCGATCGG CAATTAGCCC AAGTTTGATA ACGCTTCCCA TAGGCTTTGT GCGGAAAATT

2401 TCATGTTGCG ATTTAATTTT TTTGATGTAT TCCTCGGTGC CAGGATCCAT GTGGAATTG CTACAAAGAA AAGTSTAATC

2481 TTCGGGCAAA TCCATGGTAG GAATTGGCTT GCTGTGTTTC ATCAATTGTT CAAAAAATC CGATTTTCTA GCCATTTTGT

2561 GCAATTGTTG TTGAGTCCCG ATGAATTTAC GAGAAGGGCA TTTATCGCTA CCGAAATAGA ACAATCCAAG CGATGGGGCG

2641 TACAAAATC CTAGCTTAGC CGTATTATTC TCACTAAGC CTAGACACAC GCAATATCA TCTGTTTGT TGACAAAATC

2721 CATGGTGCCA TCAATAGGGT CTGCAATCCA ATAGGTGGGC GTATTCTAA TTTCTGTAA AGAATCCTTA TCTCTTCT

2801 CACTAAAGTA TGAATGTCT GTAAAGGAAA CATGTTTTG CAAGATTTG TTGGCGGCTA AATCTGCACT TGTAACAGGC

2881 GATCCGTCGG CTTTGTCTC GGTGGAGAAT CCGTTTTGGA TTGTTTTAAA ACCTCTTCGC CAGCAAGTGC TACAGCCCGT

2961 GTTGCATTT CTAATAAATT CATAATCATT CTTTATTCT CGAACAAAGT CAAATAATTC TCTGTATTAA AAAATAATTT

3041 TGCGGATAAA AATTAATAAT TATATATAAA ATATCTCTGC AAAAAACCA ATCAAATATT TAGTGAAATA AAAAAAATTA

3121 GATTGTAAAT TTGCCTTATG TTTTAGAGA ATACCATAAA TCATAGAAAA AATACGGGCT GGATCGAAGT AATCTGTGGC

3201 TCTATGTTTT CGGGCAAAAC CGAAGAGTTG ATTCGTAGAG TGAACGAGC CGAATTGGCT GGGCAAAAGG TAGAATCTT
<<.....R5.....<<AAGCTTAAG

HindIII

3281 TAAACCCGCA ATTGATAAAC GCTACGATGA GCAAGATGTG GTATCGCATG ATGAAAACAA AAAACAAGCA ACCCCGATTG

3361 AGGCGAGTTC TAACTTGCCC ATTTTAGCAA GCGATTGTGA TGTGGTGGG ATAGATGAGG CTCAATTCTT TGACGAAGGA

3441 ATTGTTGAGG TGCGAAATCT TTTAGCTAAT TCGGGGAAAA GAATAATTAT TGCGGGATTA GACATGGATT TTAAGGTGC
<<.....R_{ecA}O1.....<<

3521 TCCATTTGGT CCTATGCCAA ATTTAATGGC GGTAGCGGAA TATGTGACCA AAGTGCATGC AATCTGTGTG AAAACAGGGA

table 5

group	no. of chickens	Treatment			Results	
		vaccination at day 1	challenge at day 25	challenge at day 31	% of max airsac score at day 10 (safety)	% of max airsac score at day 38 (efficacy)
1	25	NDV	NDV	WT-OR aerosol	2.5	25 ^b
2	25	NDV	NDV	WT-OR aerosol	7.5	23 ^b
3	25	NDV	NDV	WT-OR aerosol	68	10 ^b
4	25	NDV	NDV	WT-OR aerosol	0	47
5	25	NDV	NDV	NDV	0	2

^b Significantly different ($p < 0.05$) compared to the controls (group 11) using two-sided Mann-Whitney U test

table 6

group	no. of chickens	Treatment			Results
		vaccination at day 1		challenge day 35	
1	15	PurD aerosol	NDV	WT-OR aerosol	no reduction
2	15	PurD aerosol	NDV	WT-OR aerosol	54% ^b
3	15	NDV	NDV	WT-OR aerosol	no reduction
4	15	MAS	NDV	WT-OR aerosol	no reduction
5	15	MAS	PurD aerosol	VT-OR aerosol	50% ^b

^b Significantly different ($p < 0.05$) compared to the controls (group 1) using two-sided Mann-Whitney U test